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# INFANTILE STIMULATION PRODUCES MILD ENHANCEMENT IN A PRIMARY HUMORAL RESPONSE OF ADULT ALBINO RATS [1]

M. A. PERSINGER AND HERMANN FALTER

*Laurentian University*

*Summary.*—Rat pups from 12 litters were handled daily, once every three days, or never touched between postnatal Days 5 and 20. At 100 days of age, 20 days after inoculation with human serum albumin, trunk blood was obtained to measure the antigen binding capacity (ABC). Rats handled as infants displayed relative immunofacilitation compared to those that had not been handled; the effect explained 7% of the variance in ABC values. In comparison, sex differences explained 14% of the variance. The results support the hypothesis that early disruptions in mother-infant bonding increase the likelihood of developing allergic responses following exposure to novel antigens during adulthood.

Rats that have been stimulated by frequent handling between birth and weaning display chronic changes in behaviour that are evident in adulthood (Denenberg, 1968). The most well-established and consistent effect of early handling is the reduction in adult "emotional" behaviour or autonomic reactivity. Because emotional lability is strongly associated with limbic (amygdaloid/hippocampal) electrical lability that modulates the release of adrenocorticotrophic hormone or ACTH (Gallager, Flannigin, King, Smith, Munro, & Littleton, 1987) from the pituitary and hence the basal levels of corticoids, an attenuation of emotionality should reduce the "set point" of circulating hormone levels.

There is evidence for this relationship. Whereas the immediate response to prolonged maternal separation is elevated serum corticosterone (Stanton, Gutierrez, & Levine, 1988), experimentally evoked elevations of corticoids are lower in adult rats handled as infants (Wiener & Levine, 1983). In light of the powerful interactions between the pituitary-adrenal-immune systems (Ader, 1981), we explored the possibility that infantile experience could cause adult modifications in primary humoral responsiveness. If the well known reciprocal relationship between levels of corticoids and immunoresponsiveness were applicable, then rats handled as infants should display immunofacilitation to a challenge relative to nonhandled rats.

A total of 36 male and 33 female Wistar albino rats were selected as subjects; they were 100 days of age at the time of blood collection. They were obtained from 12 litters (culled to 8 pups within 24 hr.) that had been housed in plastic "shoebox" cages with ¼-in. ground corn cob bedding.

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Four of the litters were handled once every third day while another four litters were handled every day between postnatal Days 5 and 20. A third group of four litters were never handled. There were two handlers; each handler interacted with the same two litters from the intermittent treatment and the same two litters from the daily handling treatment.

Handling occurred between 1300 and 1700 hr. local time. All handling procedures were as similar as possible both within and between handlers. Each pup's dorsal and genital surfaces were stroked for 2 min. The pup was then placed in a separate cage (Persinger, 1980); total time away from the mother and nest was not more than 20 min. At 21 days of age the rats were weaned and placed in groups of 3 rats of the same sex within standard wire cages. Colony temperatures were maintained automatically within a range of 20 to 22°C; the light:dark 12:12 cycle was automatically controlled, with the onset at 0800 LT.

Over four successive days, when the rats were 80 days of age, a total of 22 rats that had not been handled, 24 rats that had been handled intermittently and 23 rats that had been handled daily during the preweaned period (36 males; 33 females) were injected subcutaneously in the right flank with 0.1 cc of 10 $\mu$g/ml of human serum albumin (HSA) and then returned immediately to the home cage. Twenty (20) days later (four separate days) each rat was carried quickly (1 min.) and singly into the operating room and decapitated within 20 sec.; trunk blood was allowed to clot for 15 min. A code number was given so that the treatment condition could not be determined by the technician who completed the chemical analyses (Falter, Reid, & Persinger, 1991). All animals displayed sera that contained more than 10% precipitate and were identified as responders.

Statistical analyses involved SPSS$^X$ software on a VAX 4000 computer. Given the range and moderate skewness of the ABC scores, square-root transformations were completed. Three-way analyses ($3 \times 2 \times 2$) of variance (treatment, gender, and handler) for the ABC values indicated that only the sex ($F_{1,57} = 12.37$, $p = .001$, *eta* = 0.38) and handling differences ($F_{2,57} = 3.11$, $p = .05$, *eta* = 0.27) were statistically significant. All three factors explained only 25% of the variance in ABC values.

Means and standard deviations (in parentheses) for the square root ABCs for the never-handled, handled every three days, and handled daily groups were 1.3 (0.9), 1.8 (0.8), and 1.9 (0.6), respectively. Duncan's *post hoc* analyses indicated that the major source of the difference was the relative immunofacilitation of the litters that had been handled daily compared to the litters that had never been handled. Mean ABC values for males and females were 1.4 (0.8) and 2.0 (0.7), respectively. Correlations between ABC measures and body weight, for each sex separately, were not statistically significant.

Our results are compatible with the general concept that early handling in rodents can produce effects that continue into adulthood; the data support the specific hypothesis that early experience can differentially affect humoral immunity. Considering the observation that early handling results in reduced, stimulus-evoked corticosteroid levels in adult rats (Wiener & Levine, 1983), the relative adult immunofacilitation in our handled rats is expected.

The absolute strength of the effect is quite small and may have more epidemiological implication than utility to pursue specific behavioral-neuroimmunological interactions. However, the lack of statistically significant differences in ABC between adult rats that had been handled once every three days or every day as pups indicates that the infantile handling effect upon adult immunoresponsiveness is quite potent and requires very few episodes (perhaps even less than six) to be elicited.

Early handling and infantile stimulation have been traditionally considered a generalized form of Selye "stress." One can also define the effects in terms of the behavioral operations. Multiple interferences with maternal-offspring interaction lead to disruptions in maternal-infant bonding. Such disruptions could produce changes in microstructural organization of the limbic system, especially the anterior cingulate and amygdaloid regions, that would produce chronic elevations in vigilance or arousal. Correlative mild increases in hippocampal-amygdaloid activity, especially within novel settings (where "new" antigens are more likely to be encountered), would be associated with the nonlinear decreases in circulating ACTH levels and hence immunofacilitation; the net effect would be an exaggerated response to the mundane but organismically "new" antigen. If this interpretation is generalizable to human beings, then older children and adults who have had comparable early experiences should display both increased risk to and incidence of specific and "nonspecific" allergies.

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